TECHNICAL MANUAL

Wizard® HMW DNA Extraction Kit

Instructions for Use of Product
A2920

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TM604

Wizard® HMW DNA Extraction Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Wizard® HMW DNA Extraction Kit is designed for isolation of high-molecular-weight DNA from white blood cells (Section 3.A), tissue culture cells (Section 3.B), plant tissue (Section 3.C), and Gram-positive and Gram-negative bacteria (Section 3.D). Table 1 lists the recommended starting material from each of these sources.

DNA purified with this system is particularly suitable for long-read sequencing platforms when the protocol is closely followed and DNA handled carefully. Deviating from the recommended protocol, particularly the pipette-mixing or vortexing instructions, will cause mechanical shearing of HMW DNA and decrease average fragment sizes. Such DNA shearing will negatively affect performance in long-read sequencing applications. To maximize genomic DNA size, the protocols for this kit incorporate the use of commonly available 1,000µl wide-bore pipette tips. Using these wide-bore tips minimizes mechanical damage that may occur during required mixing steps. While these wide-bore tips will yield larger average fragment sizes, be careful during mixing steps and when handling purified DNA after rehydration. Avoid vigorous vortexing and use 200µl wide-bore tips to homogenize rehydrated DNA samples, if necessary.

Note: If wide-bore pipettes are unavailable, you can still extract HMW genomic DNA by substituting an alternate mixing strategy described in the protocol. This alternative combines the use of standard-bore pipette tips with gentle vortexing to replace wide-bore pipette mixing. While there is a slight reduction in average size of purified DNA, the yield may increase.

1. Description (continued)

Table 1. Recommendations for Sample Input.

Species and Material	Suggested Starting Material
Human Whole Blood (Yield varies with white cell count)	300µl
Tissue Culture Cells	1–3 × 10^6 cells
Plant Tissue	40mg
Gram-Negative Bacteria *Escherichia coli* JM109 overnight culture, ~3 × 10^9 cells/ml	1ml
Gram-Positive Bacteria *Listeria innocua* overnight culture	1ml

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Wizard® HMW DNA Extraction Kit	**50 isolations**	**A2920**

Each system contains sufficient reagents for 50 miniprep isolations of genomic DNA from a variety of sample types. Includes:

- 50ml HMW Blood Lysis Buffer
- 25ml HMW Lysis Buffer A
- 1.0ml Proteinase K Solution
- 25ml Protein Precipitation Solution
- 50ml DNA Rehydration Solution
- 250µl RNase A Solution

Storage Conditions: Store the Wizard® HMW DNA Extraction Kit at room temperature (15–30°C). See product label for expiration date.

3. Protocols for HMW Genomic DNA Isolation

3.A. Isolating HMW DNA from Whole Blood

We tested the purification of genomic DNA from fresh whole blood collected in EDTA, heparin and citrate anticoagulant tubes and detected no adverse effects during subsequent DNA manipulations, including PCR (2).

This protocol has been designed and tested for 300µl blood samples. Larger volumes may be processed by first harvesting the white blood cells, then resuspending and pooling up to three resuspended white blood cell pellets prior to treatment with HMW Lysis Buffer A. The yield of genomic DNA will vary, depending on the quantity of white blood cells present. Frozen blood can be used in the following protocols, but yield may be lower than that obtained using fresh blood and require additional HMW Blood Lysis Buffer.

When handling blood samples, follow the recommended procedures for biohazardous materials at your institution or see reference 3.

Materials to Be Supplied by the User

- sterile ClickFit Microtubes, 1.5ml
- standard 1.5ml microcentrifuge tubes
- phosphate-buffered saline (PBS) or TE buffer
- 37°C water bath or dry incubator/shaker
- 56°C water bath or dry incubator/shaker
- isopropanol, room temperature
- 70% ethanol, room temperature
- wide-bore pipette tips (1,000µl and 200µl)
- **optional:** 65°C water bath (for rapid DNA rehydration)

1. Add 900µl of HMW Blood Lysis Buffer to a sterile ClickFit Microtube, 1.5ml.

Blood must be collected in EDTA, heparin or citrate anticoagulant tubes to prevent clotting.

2. Gently rock the tube of blood until thoroughly mixed; then transfer 300µl of blood to the tube containing the HMW Blood Lysis Buffer. Invert the tube 5–6 times to mix.
3. Incubate the mixture for 10 minutes at room temperature (invert 2–3 times once during the incubation) to lyse the red blood cells. Centrifuge at 13,000–16,000 × *g* for 20 seconds at room temperature.
4. Remove and discard as much supernatant as possible without disturbing the visible white pellet. Approximately 10–20µl of residual liquid may remain in the 1.5ml tube.
5. If blood sample has been frozen, repeat Steps 1–4 until pellet is white. There may be some DNA loss from frozen samples.

   Note: Some red blood cells or cell debris may be visible along with the white blood cells. If the pellet appears bright red, add an additional aliquot of HMW Blood Lysis Buffer after removing the supernatant above the cell pellet, and then repeat Steps 3–4.

3.A. Isolating HMW DNA from Whole Blood (continued)

6. Add 100µl of PBS to the cell pellet and vortex the tube vigorously until the white blood cells are resuspended (10–15 seconds). If pooling multiple tubes, transfer the 100µl of suspension to the additional tubes in sequence, vortexing each to suspend the pellets.

! Completely resuspend the white blood cells to obtain efficient cell lysis.

7. Add 500µl of HMW Lysis Buffer A. Using 1,000µl wide bore pipette tips, mix the solution five times to lyse the white blood cells. Draw the tube contents slowly from the bottom of the tube, then expel the lysate rapidly down the side of the tube. The solution should become very viscous. Do **not** pipette more than five times to avoid DNA shearing.

   Notes:

   1. If wide-bore pipette tips are unavailable, use standard 1,000µl pipette tips for mixing, gently aspirating and expelling after adding HMW Lysis Buffer A.
   2. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps are disappear.

8. Add 3.0µl of RNase A Solution to the lysate and mix the sample by inverting the tube 5–7 times. Incubate the mixture at 37°C for 15 minutes.

9. Add 20µl of Proteinase K Solution to each lysate and mix the sample by inverting the tube 10 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes or chill on ice 1 minute.

10. Add 200µl of Protein Precipitation Solution to the lysate. Using 1,000µl wide bore pipette tips, aspirate to mix the solution five times. Draw the tube contents from the bottom of the tube, then expel the lysate rapidly down the side of the tube. Do not mix by pipette more than five times to avoid DNA shearing. Small protein clumps may be visible after mixing.

    Note: If wide-bore pipette tips are unavailable, vortex for 5 seconds to mix lysate and Protein Precipitation Solution.

11. Centrifuge at 13,000–16,000 × *g* for 5 minutes at room temperature. A dark brown protein pellet should be visible. If any unpelleted debris is visible, repeat the centrifugation step. If no pellet is observed, refer to Section 4, Troubleshooting.

12. Slowly transfer the supernatant to a clean 1.5ml microcentrifuge by decanting the sample into a tube containing 600µl of room-temperature isopropanol.

    Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

13. Gently mix the solution by gently inverting the tube eight (8) times. Allow 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may become visible. Centrifuge at 13,000–16,000 × *g* for 2 minutes at room temperature. The DNA will be visible as a small white pellet.

14. Decant the supernatant and add 600µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge at 13,000–16,000 × *g* for 2 minutes at room temperature.

15. Carefully aspirate the ethanol. Standard pipette tips may be used for this step. The DNA pellet is very loose at this point so carefully avoid disturbing or aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

16. Add 100µl of DNA Rehydration Solution to the tube. Do **not** vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Alternatively, incubate the purified DNA at 65°C for 1 hour, periodically mixing the solution by gently tapping the tube.

17. If DNA appears non-homogeneous (e.g., undissolved pellet is still visible), mix with 200µl wide-bore pipette tips. Store the DNA at 2–8°C.

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3.B. Isolating HMW DNA from Tissue Culture Cells

Materials to Be Supplied by the User

- sterile ClickFit Microtubes, 1.5ml
- standard 1.5ml microcentrifuge tubes
- phosphate-buffered saline (PBS)
- 37°C water bath or dry incubator/shaker
- 56°C water bath or dry incubator/shaker
- isopropanol, room temperature
- 70% ethanol, room temperature
- wide-bore pipette tips (1,000µl and 200µl)
- **optional:** 65°C water bath, (for rapid DNA rehydration)

1. Harvest tissue culture cells, and transfer them to a 1.5ml microcentrifuge tube. For adherent cells, trypsinize the cells before harvesting.

   Note: Do not exceed 3×10^6 cells for each isolation.

2. Centrifuge at 13,000–16,000 × *g* for 20 seconds to pellet the cells.

3. Remove the supernatant, leaving behind the cell pellet plus 10–50µl of residual liquid.

4. Wash the cells by adding 200µl of PBS and vortexing vigorously to resuspend cells. Centrifuge as instructed in Step 2, and remove the PBS.

5. Resuspend the pellet in 100µl of PBS by vigorous vortexing.

6. Add 500µl of HMW Lysis Buffer A. Using 1,000µl wide bore pipette tips, mix the solution five times to lyse the cells. Draw the tube contents slowly from the bottom of the tube, then expel the lysate rapidly down the side of the tube. The solution should become very viscous. Do **not** pipette more than five times to avoid DNA shearing.

   Notes:

   1. If wide-bore pipette tips are unavailable, use standard 1,000µl pipette tips for mixing the HMW Lysis Buffer A.
   2. If clumps of cells are visible after mixing, incubate the solution at 37°C until the clumps disappear.

7. Add 3.0µl of RNase A Solution to the nuclear lysate and mix the sample by inverting the tube 5–7 times. Incubate the mixture at 37°C for 15 minutes.

8. Add 20µl of Proteinase K Solution to each and mix the sample by inverting the tube 10 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes or chill on ice 1 minute.

9. Add 200µl of Protein Precipitation Solution to the nuclear lysate. Using 1,000µl wide bore pipette tips, mix the solution 5 times. Draw the tube contents from the bottom of the tube, then expel the lysate rapidly down the side of the tube. Small protein clumps may be visible after mixing. Incubate on ice for 5 minutes.

   Note: If wide-bore pipette tips are unavailable, vortex lysate and Protein Precipitation Solution for 5 seconds. Do not tip mix.

10. Centrifuge at 13,000–16,000 × *g* for 10 minutes at room temperature. A whitish protein pellet should be visible. If any unpelleted debris is visible, repeat the centrifugation step. If no pellet is observed, refer to Section 4, Troubleshooting.

11. Slowly transfer the supernatant to a clean 1.5ml microcentrifuge by decanting the sample into a tube containing 600µl of room-temperature isopropanol.

    Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

12. Gently mix the solution by gently inverting the tube eight times. Incubate 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may form a visible mass.

13. Centrifuge at 13,000–16,000 × *g* for 2 minutes at room temperature The DNA will be visible as a small white pellet.

14. Decant the supernatant and add 600µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge as instructed in Step 13.

15. Carefully aspirate the ethanol. Standard pipette tips may be used for this step. The DNA pellet is very loose at this point so carefully avoid disturbing or aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-drythe pellet for 10–15 minutes.

16. Add 100µl of DNA Rehydration Solution to the tube. Do **not** vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Alternatively, incubate the purified DNA at 65°C for 1 hour, periodically mixing the solution by gently tapping the tube.

17. If DNA appears non-homogeneous (e.g., undissolved pellet is still visible), mix with 200µl wide-bore pipette tips. Store the DNA at 2–8°C

3.C. Isolating HMW Genomic DNA from Plant Tissue

Materials to Be Supplied by the User

- sterile 1.5ml microcentrifuge tubes
- sterile ClickFit Microtubes, 1.5ml
- phosphate-buffered saline (PBS)
- liquid nitrogen
- microcentrifuge tube pestle or mortar and pestle
- 65°C water bath
- 37°C water bath
- isopropanol, room temperature
- 70% ethanol, room temperature
- wide-bore pipette tips (1,000µl and 200µl)

1. Process leaf tissue by freezing with liquid nitrogen and grinding into a fine powder using a microcentrifuge tube pestle or a mortar and pestle. Add 40mg of this leaf powder to a 1.5ml microcentrifuge tube.
2. Add 500µl of HMW Lysis Buffer A, and vortex 1–5 seconds to wet the tissue.
3. Incubate at 65°C for 15–30 minutes.
4. Cool the lysate to room temperature for 5 minutes. Add 3µl of RNase A Solution to the sample and mix by inverting the tube 5–7 times. Incubate the mixture at 37°C for 15 minutes.
5. Add 20µl of Proteinase K Solution to each leaf sample and mix the sample by inverting the tube 10 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes or chill on ice 1 minute.
6. Centrifuge at 13,000–16,000 × g for 3 minutes at room temperature to pellet any insoluble material. Transfer the lysate to a clean 1.5ml microcentrifuge tube.
7. Add 200µl of Protein Precipitation Solution to the nuclear lysate. Using 1,000µl wide bore pipette tips, mix the solution five times. Draw the tube contents from the bottom of the tube, then expel the lysate rapidly down the side of the tube. Small protein clumps may be visible after mixing. Incubate on ice for 5 minutes.

   Note: If wide-bore pipette tips are unavailable, vortex lysate and Protein Precipitation Solution for 5 seconds. Do not tip mix.
8. Centrifuge at 13,000–16,000 × *g* for 10 minutes at room temperature. A greenish pellet should be visible. If any unpelleted debris is visible, repeat the centrifugation step.
9. Slowly transfer the supernatant to a clean 1.5ml microcentrifuge tube by decanting the sample into a tube containing 600µl of room-temperature isopropanol.

   Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.
10. Gently mix the solution by gently inverting the tube eight times. Incubate for 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may form a visible mass.

11. Centrifuge at 13,000–16,000 × *g* for 2 minutes at room temperature. The DNA may be visible as a small white pellet.
12. Decant the supernatant and add 600µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge as instructed in Step 11.
13. Discard the supernatant then repeat Step 12.
14. Carefully aspirate the ethanol. The DNA pellet is very loose at this point so carefully avoid disturbing or aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-drythe pellet for 10–15 minutes.
15. Add 100µl of DNA Rehydration Solution to the tube. Do **not** vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Alternatively, incubate the purified DNA at 65°C for 1 hour, periodically mixing the solution by gently tapping the tube.
16. If DNA appears non-homogeneous (e.g., undissolved pellet is still visible), mix with 200µl wide-bore pipette tips. Store the DNA at 2–8°C.

3.D. Isolating HMW DNA from Gram-Positive and Gram-Negative Bacteria

Purifying genomic DNA from bacteria works best when cultures are grown for 14 hours or less, ideally in exponential growth phase. Cultures grown for longer tend to produce large amounts of protein and RNA that can affect the performance and purity of the isolated genomic DNA.

Materials to Be Supplied by the User

- sterile 1.5ml microcentrifuge tubes
- sterile ClickFit Microtubes, 1.5ml
- phosphate-buffered saline (PBS)
- 80°C water bath
- 37°C water bath
- 56°C water bath
- isopropanol, room temperature
- 70% ethanol, room temperature
- wide-bore pipette tips (1,000µl and 200µl)
- **optional:** 65°C water bath (for rapid DNA rehydration)
- 10mg/ml lysozyme (Sigma Cat.# L4919; for Gram-positive bacteria)
- 10mg/ml lysostaphin (Sigma Cat.# L7386; for Gram-positive bacteria)

1. Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.
2. Centrifuge at 13,000–16,000 × *g* for 2 minutes to pellet the cells. Remove the supernatant. For Gram-positive bacteria, proceed to Step 3. For Gram-negative bacteria, resuspend the cells thoroughly in 100µl of PBS, then proceed to Step 5.
3. Add the appropriate lytic enzyme(s) to the cell pellet in a total volume of 100µl, and gently pipet to mix. This treatment will weaken the cell wall for efficient cell lysis.

   Note: For certain *Staphylococcus* species, a mixture of 60µl of 10mg/ml lysozyme and 60µl of 10mg/ml lysostaphin is required for efficient lysis. However, many Gram-positive bacterial strains (e.g., *Bacillus subtilis*, *Micrococcus luteus*, *Nocardia otitidiscaviarum*, *Rhodococcus rhodochrous* and *Brevibacterium albidium*) lyse efficiently using lysozyme alone.
4. Incubate the sample at 37°C for 30–60 minutes.
5. Add 500µl of HMW Lysis Buffer A. Using 1,000µl wide bore pipette tips, mix the solution five times to lyse the cells. Draw the tube contents slowly from the bottom of the tube, then expel the lysate rapidly down the side of the tube. The solution should become very viscous. Do **not** pipette more than five times to avoid DNA shearing.

   Note: If wide-bore pipette tips are unavailable, use standard 1,000µl pipette tips for mixing sample with HMW Lysis Buffer A.
6. If lysis appears incomplete, incubate at 80°C for 5 minutes to lyse the cells then cool to room temperature.

7. Add 3µl of RNase A Solution to the cell lysate, and mix by inverting the tube 5–7 times. Incubate the mixture at 37°C for 15 minutes.

8. Add 20µl of Proteinase K Solution to each sample and mix by inverting the tube 10 times. Incubate the mixture at 56°C for 15 minutes. Cool to room temperature for at least 5 minutes or chill on ice 1 minute.

9. Add 200µl of Protein Precipitation Solution to the cell lysate. Using 1,000µl wide bore pipette tips, mix the solution five times. Draw the tube contents from the bottom of the tube, then expel the lysate rapidly down the side of the tube. Small protein clumps may be visible after mixing. Incubate on ice for 5 minutes.

   Note: If wide-bore pipette tips are unavailable, vortex lysate and Protein Precipitation Solution for 5 seconds. Do not tip mix.

10. Centrifuge at 13,000–16,000 × *g* for 10 minutes at room temperature. A protein pellet should be visible. If any unpelleted debris is visible, repeat the centrifugation step. If no pellet is observed, refer to Section 4, Troubleshooting.

11. Slowly transfer the supernatant to a clean 1.5ml microcentrifuge tube by decanting the sample into a tube containing 600µl of room-temperature isopropanol.

    Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

12. Gently mix the solution by gently inverting the tube eight times. Incubate 1 minute at room temperature and repeat the inversion. White thread-like strands of DNA may form a visible mass.

13. Centrifuge at 13,000–16,000 × *g* for 2 minutes at room temperature. The DNA will be visible as a small white pellet.

14. Decant the supernatant and add 600µl of room temperature 70% ethanol to the DNA. Gently invert the tube several times to wash the DNA pellet and the sides of the microcentrifuge tube. Centrifuge as instructed in Step 14.

15. Carefully aspirate the ethanol. The DNA pellet is very loose at this point so carefully avoid disturbing or aspirating the pellet into the pipette. Invert the tube on clean absorbent paper and air-dry the pellet for 10–15 minutes.

16. Add 100µl of DNA Rehydration Solution to the tube. Do **not** vortex because this will cause mechanical shearing and decrease average fragment size. Rehydrate the DNA by incubating the solution overnight at room temperature. Alternatively, incubate the purified DNA at 65°C for 1 hour, periodically mixing the solution by gently tapping the tube.

17. If DNA appears non-homogeneous (e.g., undissolved pellet is still visible), mix with 200µl wide-bore pipette tips. Store the DNA at 2–8°C.

4. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Blood clots present in blood samples	The collection tube may have been stored improperly; the blood was not thoroughly mixed, or inappropriate tubes were used for drawing blood. Discard the clotted blood and draw new samples using EDTA-, heparin- or citrate-treated anticoagulant tubes.
Poor DNA yield	Blood sample may contain too few white blood cells. Draw new blood samples.
	White blood cell pellet was not resuspended thoroughly in Section 3.A, Step 5. The white blood cell pellet must be vortexed vigorously to resuspend the cells.
	Blood sample was too old. Best yields are obtained with fresh blood. Samples that have been stored at 2–8°C for more than 5 days may give reduced yields.
	The DNA pellet was lost during isopropanol precipitation. Be careful when removing the isopropanol to avoid losing the pellet.
	Sample is contaminated with RNA. DNA yield can be skewed by the presence of digested RNA when assayed spectrophotometrically. We recommend the use of DNA-specific fluorometric assays.
	Isolated genomic DNA is not homogeneous, affecting concentration determination. Purified genomic DNA commonly forms concentration gradients and can affect the concentration determination. Mix the sample by flicking the tube several times or vortex briefly. Do not mix with a pipette. Reassay the samples.
High RNA contamination	Overgrown bacterial culture. When bacterial cultures are grown longer than 14 hours (stationary phase), they are prone to high amounts of RNA during purification. Limit growth to exponential phase.
Poor separation after protein precipitation	Added too much sample. Do not exceed the recommended maximum inputs for sample type. Too much sample results in excess viscosity, which will affect separations.
Degraded DNA (<50kb in size)	Improper collection or storage of the blood sample. Obtain a new sample under the proper conditions.

Symptoms	Causes and Comments
Poor DNA yield using Gram-positive bacteria protocol	Bacterial culture grown too long. Cultures grown for an extended time contain a high proportion of cells that lyse easily upon exposure to lysostaphin treatment. Start purifications with a healthy culture.
No protein pellet	The sample was not cooled to room temperature before adding the Protein Precipitation Solution. Cool the sample to room temperature (at least 5 minutes) or chill on ice for 5 minutes, vortex for 20 seconds, centrifuge for 3 minutes at 13,000–16,000 × *g* and proceed with the protocol.
	The Protein Precipitation Solution was not thoroughly mixed with the lysate. Always mix the lysate and Protein Precipitation Solution completely.
DNA pellet difficult to dissolve	Samples may have been overdried. Rehydrate DNA by incubating for 1 hour at 65°C, and then leave the sample at room temperature or 4°C overnight. **Caution:** Do not leave the DNA at 65°C overnight.
	Samples were not mixed during the rehydration step. Remember to mix the samples periodically during the rehydration step. Use only 200µl wide-bore tips for mixing.

5. References

1. Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.
2. Beutler, E., Gelbart, T. and Kuhl, W. (1990) Interference of heparin with the polymerase chain reaction. *BioTechniques* **9**, 166.
3. U.S. Department of Labor, Occupational Safety and Health Administration (1991) Occupational exposure to bloodborne pathogens, final rule. *Federal Register* **56**, 64175.

6. Composition of Buffer

Phosphate-buffered saline (PBS) 10X (per liter)

11.5g	Na_2HPO_4
2g	KH_2PO_4
80g	NaCl
2g	KCl

Dissolve in 1 liter of sterile, deionized water. The pH of 1X PBS will be 7.4.

7. Related Products

DNA Purification Systems

Product	Size	Cat.#
ProNex® Size-Selective Purification System	10ml	NG2001
ProNex® Size-Selective Purification System	125ml	NG2002
ProNex® Size-Selective Purification System	500ml	NG2003
Maxwell® RSC Plant DNA Kit	48 preps	AS1490
ReliaPrep™ Blood gDNA Miniprep System	100 preps	A5081